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MICROBIOLOGICAL ANALYSIS OF DEBRIS FROM STS-42 IML-1 BY DIRECT PLATING OF RINSE WATERS

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Microbial analysis of air filter debris from Spacelab mission IML-1 was performed via direct plating of rinse waters on a battery of selective and nonselective nutrient agars. Microbial isolates were identified using Minitek and Biolog technologies. Twenty-four types of bacteria were recovered and classified; a similar number of fungal types was observed, but these were not identified. This procedure can provide information about the proportions of organism types present at the time of debris collection.

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TECHNICAL MEMORANDUM

MICROBIOLOGICAL ANALYSIS OF DEBRIS FROM STS-42 IML-1 BY DIRECT PLATING OF RINSE WATERS

PURPOSE, MATERIALS, AND METHODS

When STS-42 IML-1 air filter debris was processed for microbial analysis via an enrichment technique, the work reported here was undertaken as a supplemental study. Before the supplemental study began, the debris was sorted and weighed. Each sample was suspended in 5 mL of sterile water and sonicated for 15 minutes (to remove microorganisms from the debris). Approximately 3 mL of liquid were removed from each sample, enriched with nutrient, and allowed to incubate. The supplemental study began at this point. Liquids were removed from selected samples only (table 1) and spread plated (0.1 mL per plate) on various selective and nonselective agars (table 2 details attributes of agars used, tables 3 and 5 indicate which agars were used for various samples). This plating, without nutrient enrichment, occurred within 2 hours after sonication (on February 5, 1992). Only a negligible amount of liquid was available in sample C23, so liquid from this sample was not plated until the following day, at which time the sample was rinsed (without sonication) and the resulting rinse water plated. The purpose of this work was to extend the usefulness of the debris sample, since the two techniques (enrichment and more direct plating) were thought to offer different advantages for information gathering.

SS, mannitol salt, and Pseudomonas isolation agar plates were incubated at 35 °C. EMB, triple sugar iron, R2A, Staphylococcus medium 110, and tryptic soy agar plates were incubated at 28 °C. With the day of plating as day 1, plates were observed on days 2, 3, 6, and 10 (except for C23 which was observed on days 2, 5, and 9). Colony counts (or approximate counts) and limited morphological observations were recorded.

Specific identification procedures were conducted over a period of weeks. Before identification, colonies from initial plates were streaked (and sometimes restreaked) on tryptic soy agar to obtain pure cultures. Twenty-four hour cultures were used for the identification process. Sixty-four bacterial isolates were identified or classified (46 by the Minitek procedure, 11 by the Biolog procedure, and 7 by microscopic observation of gram stains). Molds and yeasts were not identified, but gross characteristics were recorded and photographs were made. In some cases, confluent molds on initial spread plates were streaked on tryptic soy agar to obtain distinct isolates.

RESULTS

Twenty-four types of bacteria were identified or classified. Table 7 lists these organisms by sample number. Representatives of 10 bacterial genera were identified. Seven species of genus *Staphylococcus* were identified. One type of yeast and approximately 20 types of mold were observed (table 8 lists mold descriptions by sample number and agar type). With different agar types, it was not always possible to determine, by gross observation, if individual isolates were identical.

Tables 3 to 6 show colony counts and identifications by agar type for avionic and cabin samples. Agar color sometimes provided useful information. A13 on mannitol turned the agar yellow (with approximately 200 colonies), but A12 on mannitol remained red (with approximately 1,000 colonies). This indicates that A13 contained pathogenic species (such as *Staphylococcus aureus*) and A12 contained "nonpathogenic" species (such as *Staphylococcus epidermidis* and *Staphylococcus hominis*). C18 on TSIA (with two fungal colonies) turned yellow, indicating acid production; A9 (with 26 bacterial colonies) remained red, indicating the absence of acid production.

Colony morphology notes were limited. The count on a particular plate was divided into morphological types only when types were distinct enough to be easily separated. Thus, when a particular plate shows only "300 W" this does not necessarily mean that only one species was present. Likewise, if the colony count table shows three morphological types for a particular plate and the identification table shows three identifications for that same plate, it does not necessarily mean that the identifications and the morphological types can be directly matched; perhaps one morphological type provided two identifications and another morphological type was not identified. It should also be stated that this was not an exhaustive study. When a particular plate shows two identifications, it should not be assumed that no other organisms were present.

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Direct plating of debris rinse water on a battery of different agars is a useful procedure. Looking at the samples which were plated on five or more agars, it is easy to judge the proportions of biotypes present. A9 shows mostly one type of mold, no gram positives, and some gram negatives. In A12, gram positives greatly outnumber gram negatives, several types of mold are present in small numbers, and gram positives appear to be mostly *S. epidermidis* and *S. hominis* rather than *S. aureus*. A13 appears to be largely *S. aureus*. C2 is not clear cut. C13 shows similar numbers of gram positives and negatives. C16 appears to be gram positives and molds. In C18, *Methylobacter* flourishes where competitors are almost absent. C22 is mostly gram positives.

Most of the identified organisms commonly inhabit humans, and it is not surprising that these should be present in air filters. However, since most of them are opportunistic pathogens, since astronauts are immunocompromised, and since it is not known how the microbial community will develop in a space station environment (will there be an increased proportion of pathogens?), it is good to be aware of the potential pathogenicities of these organisms. Appendix 1 contains excerpts from *The Prokaryotes* (the international standard for bacterial taxonomy) which provide pertinent details about the organisms identified.

Comparing bacterial identifications reported here for IML-1 filter debris (partial sample) to those reported for SLS-1 filter debris (EH32 (91-597) and ED62 (124-91)) and SLS-1 humidity condensate (EH32 (91-631)), the three samples do have some organisms in common. *Methylobacter* sp. and *Staphylococcus haemolyticus* were found in all three samples. (*Methylobacter* was also found in IML-1 humidity condensate; this will be reported in a separate document.) *Methylobacter*, easily isolated from environmental samples, has been tentatively linked to vehicular emissions and is particularly resistant to gamma-ray irradiation (see appendix 1). In addition, the following organisms were found in both IML-1 debris and SLS-1 debris: *Enterobacter agglomerans, Micrococcus luteus, Pseudomonas paucimobilis, Staphylococcus epidermidis, Staphylococcus hominis,* and *Staphylococcus aureus.* A number of types of fungi were found in both SLS-1 and IML-1. In addition, the large gram-positive rods reported here are

possibly *Bacillus*, which was reported for SLS-1. Results in this report (partial sample, direct plating) will be compared to the concurrent analysis (full sample, enrichment technique) when those data are available.

It was reported in NASA Conference Publication 3108, "Microbiology on Space Station *Freedom*," (1989) that: (1) pre/post launch air samples on the orbiter flight deck and middeck typically contained fungi such as *Aspergillus* and *Penicillium* spp., as well as bacteria like *Bacillus* and *Micrococcus* spp., while *Staphylococcus* spp. were removed in large numbers from surfaces, but not from air samples; (2) in-flight air/research animal holding facility surface/crewmember sampling during STS-51B (Spacelab 3) revealed that numbers of airborne fungi and bacteria in the Spacelab tended to increase during the flight to a maximum of 200 CFU/m^3. This publication also predicts Space Station *Freedom* conditions: "The majority of bacteria found in the S. S. *Freedom* environment will originate from human skin. During a 14-day period, approximately 2 m^2 of epidermis is shed with its associated bacteria, e.g., *Staphylococcus epidermatitis* [sic], *Micrococcus* spp. and diptheroids...Bacteria such as *S. aureus* are often spread by inhalation of skin flakes." Comparing this information to findings reported here, there is significant agreement. It is difficult, of course, to classify an air filter debris sample as an air or surface sample; it should perhaps be considered a "combination sample." *S. aureus* (regarded as a potentially serious pathogen and a major cause of mortality) was present in 7 of the 14 samples analyzed, and, according to the report quoted above, this organism is often spread by inhalation of skin flakes.

Humans were probably the source for most, if not all, of the bacteria identified. The IML-1 payload did include experiments involving insects, plants, and even microorganisms; however, containment conditions were such that these experiments probably did not contribute organisms to the debris samples (with the possible exception of the plant experiments).

The information in table 2 about selective agars is used as a guideline in interpretation of results. Laboratory practice demonstrates exceptions to the rule.

Molds and yeasts, recovered but not identified, are not insignificant factors in this work. Future analyses should focus on fungi as well as bacteria. This may necessitate the involvement of outside mycologists.

Direct plating of debris rinse water on a battery of agars followed by identification procedures can clearly provide information about the proportions of organism types present at the time of debris collection. Nutrient enrichment with an incubation period could not provide this type of information because of the selective nature of the procedure. However, enrichment may sometimes uncover organisms too stressed to be cultured by the direct plating method. It therefore seems appropriate to use both methods when debris samples are analyzed. Unfortunately, to do an exhaustive analysis, by either method, is extremely time consuming, particularly when analysis is preceded by sorting and many samples rather than a single sample must be analyzed. However, sorting of debris does appear to demonstrate association of particular species with particular materials (although the weight of the sample also is a factor in number of types of species found). Therefore, it is suggested that for microbial analysis of future debris samples: (1) the sample be sorted only on alternate missions, (2) both enrichment and direct plating techniques be used for unsorted samples, and (3) only one technique be used for sorted samples. An unsorted sample could be processed by the direct plating technique soon after landing, possibly yielding more relevant data.

Table 1. Sample descriptions and weights (in grams).

Avionics Air Filter Samples

A3	latex ? pieces 0.15
A8	nonmetallic, crystal appear 0.02
A9	plastic pieces 0.18
A12	remaining misc debris 0.26
A13	hair 0.03

Cabin Air Filter Samples

C2	food pieces 0.14
C6	plastics 0.62
C8	metal pieces 1.62
C11	latex ? pieces 0.05
C13	remaining misc debris 0.66
C16	red rubber pieces 0.08
C18	hair 0.02
C22	nonmetallic, crystal appear 0.04
C23	lint 0.94

Note: After the debris was sorted and prepared for microbial analysis via an enrichment technique, selected samples only (the 14 listed) were used in this study; "remaining misc debris" should be viewed in this context. The 14 samples selected were summarily judged most likely to yield significant microbial growth.

Table 2. Agar descriptions.

SS Agar
Intended use: a highly selective plating medium used for isolating *Salmonella* and some *Shigella*.
Prepared plates: red-orange

Mannitol Salt Agar (Mann)
Intended use: a selective medium used for the isolation of pathogenic staphylococci.
Prepared plates: red
Agar color change: pathogenic staphylococci turn the agar yellow; nonpathogenic staphylococci do not change the agar color.

Pseudomonas Isolation Agar (PI)
Intended use: a selective medium for the isolation of *Pseudomonas*. (By verbal communication with Difco representative, only *Pseudomonas aeruginosa* grows on this agar.)
Prepared plates: light amber

EMB Agar
Intended use: a differential plating medium recommended for the detection and isolation of the gram-negative enteric bacteria.
Prepared plates: purple with greenish orange cast
Dark centers: indicate lactose positive colonies.
Mucoid pink colonies: indicate *Enterobacter*.

Triple Sugar Iron Agar (TSIA)
Intended use: recommended for the identification of gram-negative enteric bacilli based on the fermentation of dextrose, lactose, and sucrose and for hydrogen sulfide production.
Prepared plates: red.
Agar color change: fermentation of the sugars, resulting in acid production, is detected by the phenol red indicator. The color changes, yellow for acid production and red for alkalinization, are striking.

R2A Agar
Intended use: a low nutrient medium for performing the heterotrophic plate count of treated potable water.
Prepared plates: colorless

Staphylococcus Medium 110 (S-110)
Intended use: Due to its high concentration of sodium chloride, this is a selective culture medium for the isolation of pathogenic strains of *Staphylococcus*. It is also well suited for pigment formation.
Prepared plates: light amber
Pigment production: *S. aureus* is positive for pigment production; *S. epidermidis* is negative for pigment production.

Tryptic Soy Agar (TSA)
Intended use: a general purpose medium used with or without blood or other enrichment for isolating an cultivating a variety of fastidious microorganisms.
Prepared plates: light amber

Note: This information is taken from the *Difco Manual*, Tenth Edition, Difco Laboratories, Detroit.

Table 3. Colony counts by agar type, avionics sample.

Sample	Bacteria							Molds
	SS	MANN	PI	EMB	TSIA	R2A	S-110	
A3		0				2-W		R2A
A8						100-YG 20-WT 5-WO		R2A
A9	0	0	0	50-CDC 13-CWE 10-P	24-P 2-Y	33-WO		SS EMB R2A
A12	0	1,000-WT 35-WO 8-YG	0	26-B 6-PDC	20-WO 25-WT	1,000-W 30-W 20-YL 60-WO	10-Y 15-W	MANN EMB TSIA R2A S-110
A13	0	200-YO	0	300-B	10-W	300-WT		

Large counts are approximate. The "Molds" column lists the agars on which molds grew; for colony counts and descriptions, see table 8.

W = white, WT = white, translucent; WO = white, opaque
Y = yellow; YG = gold-yellow; YL = light yellow
CDC = colorless, dark center; CWE = colorless, wavy edge
P = pink; PDC = pink, dark center
B = burgundy

At the time of plating, some agar plate types were in short supply; therefore, samples summarily judged least likely (of these 14 samples) to yield significant microbial growth were not plated comprehensively.

Table 4. Identifications by agar type, avionics sample.

Sample	SS	MANN	PI	EMB	TSIA	R2A	S-110
A3							
A8						*S. aureus* *S. aureus* *S. epi.* G+ rod	
A9				*E. agglom.* *P. f/p*	*E. agglom.* *P. agglom.*	*E. agglom.*	
A12				*E. agglom.* *E. agglom.* *E. agglom.* *P. f/p* *P. f/p*	*E. agglom.* *E. agglom.* *S. cohnii* *S. homin.*	*E. agglom.* *S. aureus* *S. homin.*	*S. epi.* *S. homin.*
A13				*S. aureus* *S. aureus*		*S. aureus* *S. aureus*	

Table 5. Colony counts by agar type, cabin sample.

Sample	Bacteria								Molds
	SS	MANN	PI	EMB	TSIA	R2A	S-110	TSA	
C2	0	12-WT 6-P 3-YL	0		29-WT	100-WT 50-WO			TSIA R2A
C6						53-WT 15-YL 4-YG			R2A
C8		0			0				TSIA
C11		1-YL	0		0	1-WO			R2A
C13	0	200-WO 20-YG	0	100-B 6-BWE 1-P	90-W	300-W	300-W 30-YG		SS MANN EMB TSIA R2A
C16	0	7-WT	0	0		6-WO 1-YL 1-YG	1-WT		EMB
C18	0	0	0	CONFL	0	6-WT 5-WO	0		R2A TSIA
C22	0	57-WT	0	2-B	2-W	56-YL 4-W			TSIA R2A
C23	TN-WT 20-P	300-WO 40-YG 20-WT			TN-W			TN-W	MANN

Large counts are approximate. The "Molds" column lists the agars on which molds grew; for colony counts and descriptions, see table 8.

W = white, WT = white, translucent; WO = white, opaque
Y = yellow; YG = gold-yellow; YL = light yellow
CDC = colorless, dark center; CWE = colorless, wavy edge
P = pink; PDC = pink, dark center
B = burgundy; BWE = burgundy, wavy edge

TN = too numerous to count
CONFL = confluent

At the time of plating, some agar plate types were in short supply; therefore, samples summarily judges least likely (of these 14 samples) to yield significant microbial growth were not plated comprehensively.

Table 6. Identifications by agar type, cabin sample.

Sample	SS	MANN	PI	EMB	TSIA	R2A	S-110	TSA
C2					*P. vulgar.*	*M. roseus* *S. aureus* *S. cohnii* *S. homin.* G3377172		
C6						*P. pauci.* *S. aureus* *S. epi.* *S. sapro.* G0242300		
C8								
C11		*S. homin.*						
C13		*M. luteus* *S. aureus* *S. aureus*			*P. pseudo.* *P. pseudo.* *Shigella* *Shigella* *S. aureus* G3276742 G3276742 G3376302			
C16						*M. luteus* *S. haemo.*		
C18				*Methylob.*		G+ rod		
C22		*S. haemo.*				*M. luteus* *S. homin.* *S. warneri*		
C23	*P. f/p*	*S. aureus* *S. haemo.*			*K. ozaenae* *P. f/p*			*A. lwoffi*

Table 7. Microbial isolates (by sample number) from STS-42 IML-1 filter debris

A3 (latex ? pieces)
Bacteria: None
Molds: 1 type
(See table 8 for mold descriptions)

A8 (nonmetallic, crystal appear)
Staphylococcus aureus
Staphylococcus epidermidis
Large gram-positive rod
Molds: 1 type

A9 (plastic pieces)
Enterobacter agglomerans
Pseudomonas fluorescens/putida
Pantoea agglomerans
Molds: 3 types

A12 (remaining misc debris)
Enterobacter agglomerans
Pseudomonas fluorescens/putida
Staphylococcus aureus
Staphylococcus cohnii
Staphylococcus epidermidis
Staphylococcus hominis
Molds: 6 types

A13 (hair)
Staphylococcus aureus
Molds: None

C2 (food pieces)
Micrococcus roseus
Proteus vulgaris
Staphylococcus aureus
Staphylococcus cohnii
Staphylococcus hominis
Gram-positive sphere 3377172
(unidentified by Minitek)
Molds: 3 types

C6 (plastics)
Pseudomonas paucimobilis
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus saprophyticus
Gram-positive sphere 0242300
(unidentified by Minitek)
Molds: 2 types
Yeast: 1 type (unidentified)

C8 (metal pieces)
Bacteria: None
Molds: 1 type

C11 (latex ? pieces)
Staphylococcus hominis
Molds: 4 types

C13 (remaining misc debris)
Pseudomonas pseudoalcaligenes
Shigella species
Staphylococcus aureus
Gram-positive sphere 3276742
(unidentified by Minitek)
Gram-positive sphere 3376302
(unidentified by Minitek)
Molds: 8 types

C16 (red rubber pieces)
Micrococcus luteus
Staphylococcus haemolyticus
Molds: 1 type

C18 (hair)
Methylobacterium species subgroup B
Large gram-positive rod
Molds: 4 types

C22 (nonmetallic, crystal appear)
Micrococcus luteus
Staphylococcus haemolyticus
Staphylococcus hominis
Staphylococcus warneri
Molds: 2 types

C23 (lint)
Acinetobacter lwoffi
Pseudomonas fluorescens/putida
Staphylococcus aureus
Staphylococcus haemolyticus
Klebsiella ozaenae
Molds: 3 types

Table 8. Molds—gross characteristics (colony count in parentheses).

A3	R2A	white (2)
A8	R2A	cream, moist (1)
A9	SS	pink, raised, moist (1)
	EMB	white-pink (4)
	R2A	gray center, pink-orange edge (150)
A12	Mann	gray (1)
	EMB	pink (4)
		yellow center, pink edge, white in between (2)
		white, moist, wavy edge (1)
	TSIA	green/beige/white (2)
	R2A	gray center, pink-orange edge (15)
	S110	gray (1)
		gray-green-white (1)
C2	TSIA	fuzzy chartreuse (2)
	R2A	gray center, pink-orange edge (11)
		white, delicate thin branching (9)
C6	R2A	olive green (2)
		gray center, pink-orange edge (8)
C8	TSIA	fuzzy chartreuse (1)
C11	R2A	dark green center, white edge (1)
		moist white, translucent (1)
		moist white, opaque (1)
		dark red, dry (1)
C13	SS	light pink-white (2)
	Mann	gray-white (1)
	EMB	green (2)
	TSIA	gray (7)
		white, dry (confluent)
	R2A	green, dry (confluent)
		orange (confluent)
		white (confluent)
C16	EMB	green-gray (covered)
C18	R2A	cream, moist (1)
		white, smooth (3)
	TSIA	gray-white (1)
		cream, moist (1)
C22	TSIA	white with beige center, radial lines (1)
	R2A	light gray (2)
C23	Mann	white (7)
	TSIA	pink (1)
		yellow (1)
		white (1)

APPENDIX 1. INFORMATION ABOUT MICROORGANISMS IDENTIFIED IN STS-42 IML-1 DEBRIS SAMPLES

With permission from Springer-Verlag, the excerpts below are taken from *The Prokaryotes, Second Edition, A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Applications*, edited by Albert Balows, Hans G. Truper, Martin Dworkin, Wim Harder, and Karl-Heinz Schleifer, published by Springer-Verlag, New York. These volumes should be consulted for primary references cited by chapter authors.

Chapter 23 The Denitrifying Prokaryotes

Walter G. Zumft

Taxon or bacterial group	Habitat
Pseudomonas fluorescens	Soil, water, plants, humans (clinical isolate)
Pseudomonas pseudoalcaligenes	Isolates from natural materials and clinical specimens

Chapter 56 The Genus *Micrococcus*

Miloslav Kocur, Wesley E. Kloos, and Karl-Heinz Schleifer

Mammalian skin is now considered as primary habitat of micrococci.

Human skin is a rich source of micrococci. It was shown that 96 percent of 115 people living in 18 different states in the USA carried cutaneous populations of micrococci. The percentages of individuals carrying various *Micrococcus* species were as follows: *M. luteus*, 90 percent; ... *M. roseus*, 15 percent ... Populations of *M. luteus* were usually relatively large and this organism was isolated on the average, from 51 percent of the different skin sites sampled of individuals carrying this species.

The data about possible pathogenicity of micrococci for humans are very poor and controversial. Micrococci, however, may be considered as opportunistic pathogens, particularly in view of the increasing number of immunocompromised patients.

A case of septic shock caused by *Micrococcus luteus* was described by Albertson et al. (1978). *M. luteus* was reported in a case of cavitating pneumonia of an immunosuppressed patient and in case of meningitidis [sic].

Chapter 63 The Genus *Staphylococcus*

Wesley E. Kloos, Karl-Heinz Schleifer, and Friedrich Gotz

Skin as a Habitat

Staphylococci are widespread in nature, though they are found more consistently and in denser populations on the skin, skin glands, and mucous membranes of mammals and birds.

Flies of the genera *Musca, Fannia,* and *Stomoxys* are commonly found in human and/or animal habitations, can carry populations of staphlococci and appear to be significant vectors of these organisms in an epizootiological chain.

Host Range

S. epidermidis is the most prevalent and persistent *Staphylococcus* species on human skin. . . *S. hominis* is also prevalent on human skin. Its population size is usually second or equal to *S. epidermidis* on skin sites where apocrine glands are numerous. . . *S. haemolyticus* shares many of the habitats of *S. hominis,* but it is usually found in smaller populations. Some individuals may carry unusually large populations of *S. haemolyticus*. . . *S. warneri* is found usually in small numbers on human skin, though a few individuals may carry unusually large populations. . . *S. warneri* is a major species on nonhuman primates.

S. aureus is a major species of primates, though specific ecovars or biotypes can be found occasionally living on different domestic animals or birds. . . On humans, *S. aureus* demonstrates a niche preference for the anterior nares, especially in the adult. Here it can exist as a resident or as a transient member of the normal flora.

Species of the *S. saprophyticus* species group demonstrate a variety of host ranges from humans to lower mammals and birds. . . Those species found most frequently on primates include *S. saprophyticus, S. cohnii,* and *S. xylosus. S. saprophyticus* is found usually in small, transient populations on the skin of human or other primates. This species possesses surface properties that allow it to adhere readily to urogenital cells, which ultimately may lead to urinary tract infections. It is also sometimes isolated from lower mammals and environmental sources. *S. cohnii* is found as a temporary resident or transient on human skin.

Opportunistic Pathogens

The coagulase-positive species *S. aureus* (and others) are regarded as potentially serious pathogens. *S. aureus,* since its early discovery as an opportunistic pathogen, continues to be a major cause of mortality and is responsible for a variety of infections. . . Methicillin-resistant *S. aureus* strains have emerged in the 1980's as a major clinical and epidemiological problem in hospitals.

The coagulase-negative staphylococcal species constitute a major component of the normal microflora of the human, and for this reason have generally been regarded as saprophytes or organisms with no or very low virulence. However, over the last two decades there has been an increase in the documentation of infections due to coagulase-negative staphylococci, especially with the species *S. epidermidis*. . . Of the 13 coagulase-negative species recognized from humans, *S. epidermidis* appears to have the greatest pathogenic potential and adaptive diversity.

Certain other coagulase-negative species have been associated with infections in humans and animals. *S. haemoloyticus* is the second most frequently encountered species of this group found in human clinical infections. . . *S. saprophyticus* is an important opportunistic pathogen in human urinary tract infections.

Several other coagulase-negative species have been implicated at low incidence in a variety of human infections. In most cases, patients with these infections had predisposing or underlying diseases that caused intensive changes in the immune system and had also experienced surgery or intravascular

manipulations. *S. warneri* has been, on occasion, the etiologic agent of vertebral osteomyelitis, native valve endocarditis, and urinary tract infections in males and females. . . *S. hominis* has been associated with human endocarditis, peritonitis, septicemia, and arthritis.

Chapter 117 The Genus *Methylobacterium*

Peter N. Green

The genus *Methylobacterium* is composed of a variety of pink-pigmented facultatively methylotrophic (PPFM) bacteria which are capable of growth on one-carbon compounds such as formate, formaldehyde, and methanol as sole source of carbon and energy as well as on a wide range of multicarbon growth substrates.

Members of the genus *Methylobacterium* are ubiquitous in nature and are thus found in a variety of habitats, including soil, dust, freshwater, lake sediments, leaf surfaces and nodules, rice grains, air, hospital environments, and as contaminants in various products and processes, e.g., in pharmaceutical preparations such as face creams. . . Our identification service has also had isolates from pure water users such as silicon chip manufacturers. . .

In addition, their ability to resist a certain degree of desiccation and to scavenge trace amounts of nitrogen and carbon make them well suited for survival in stressful environments. . .

The ease with which PPFM strains can be isolated from environmental samples, and their albeit-tentative link with vehicular emissions, suggests possible uses for these organisms as environmental indicators. . .

In addition, several PPFM strains have exhibited resistance to gamma-ray irradiation 10 to 40 times higher than that tolerated by several other gram-negative bacteria examined. . . This resistance, coupled with their easily identifiable pigmented colonies, may make some PPFM strains suitable candidates for irradiation-quality-control monitoring in the food and packaging industries.

Chapter 141 Introduction to the Family *Enterobacteriaceae*

Don J. Brenner

Klebsiella

Klebsiella pneumoniae, Klebsiella ozaenae, and *Klebsiella rhinoscleromatis* have long been known to be a single genospecies. Orskov (1984) proposed that they be considered as subspecies of *K. pneumoniae* (*K pneumoniae* subsp. *pneumoniae, K. pneumoniae* subsp. *ozaenae,* and *K. pneumoniae* subsp. *rhinoscleromatis*) but that only the species names be used in clinical laboratories.

Chapter 145 The Genus *Shigella*

Haruo Watanabe and Noboru Okamura

Shigella is a pathogen that causes bacillary dysentery in humans, and is considered to have a narrow distribution in the natural environment, inhabiting mainly the intestinal tract. . . The members of

the genus *Shigella* are not specially resistant to the physical or chemical antimicrobial agents such as heat, disinfectants, or antiseptics.

Chapter 147 The Genus *Klebsiella*

Francine Grimont, Patrick A. D. Grimont, and Claude Richard

Klebsiella spp. are often found in a variety of environmental situations, such as soil, vegetation, or water. . .

Klebsiella can frequently be isolated from the root surfaces of various plants. . .

Klebsiella spp. may be found in human feces. A survey of the presence of *Klebsiella* in urban residents (not associated with a hospital environment), hospital personnel, and newly admitted patients showed that 30 to 37 percent of individuals carried *Klebsiella* . . . , including 29 to 35 percent fecal carriage and 3 to 4 percent throat carriage. . . *Klebsiella* spp. can cause human diseases, ranging from asymptomatic colonization of the intestinal, urinary, or respiratory tract to fatal septicemia, and are widely recognized as important opportunistic pathogens in hospital patients. . .

K. pneumoniae subsp. *ozaenae* is considered to be associated with ozena, an atrophic rhinitis. . . In the past 10 years, several reports have stated that *K. ozaenae* (now *K. pneumoniae* subsp. *ozaenae*) may cause invasive infections, especially in immunosuppressed hosts, bacteremia with or without meningitis, otitis, mastoiditis, urinary tract infections, wound infections, corneal ulcers, pneumonia, and brain abscesses.

Chapter 148 The Genus *Enterobacter*

Francine Grimont and Patrick A.D. Grimont

Enterobacter species are found in the natural environment in habitats such as water, sewage, vegetables, and soil. Before the widespread use of antibiotics, *Enterobacter* species were rarely found as pathogens, but these organisms are now increasingly encountered, causing nosocomial infections such as urinary tract infections and bacteremia. . .

Older reports on *Klebsiella-Enterobacter* infections were taxonomically imprecise. Some laboratories used one name (*Enterobacter*) for an organism isolated from urine and another name (*Klebsiella*) for the identical organism isolated from sputum. Other laboratories based the identification on the colony type: mucoid colonies were designated *Klebsiella* and nonmucoid colonies were reported as *Enterobacter.*

The *Enterobacter agglomerans* complex is ubiquitous in the environment. This species predominates on the leaf and bract of pre- and postsenescent cotton plants. . .

E. agglomerans may occur in clinical samples (blood, wounds, sputum, urine), often with dubious clinical significance. . . In an Ohio hospital, *E. agglomerans* accounted for 4 of 58 episodes of *Enterobacter* bacteremia and one of 42 nosocomial bacteremia. In 1970, *E. agglomerans* was implicated in a nationwide outbreak of septicemia caused by contaminated closures on bottles of infusion fluids in the United States. Twenty-five hospitals were involved, with 378 cases and 40 deaths.

Chapter 151 The Genera *Proteus, Providencia,* and *Morganella*

John L. Penner

The Genus *Proteus*

Although well recognized as opportunistic infectious agents of the urinary tract, *P. mirabilis* and *P. vulgaris* may cause, under suitable conditions, infections of the respiratory tract, wounds, burns, skin, eyes, nose, and throat.

Chapter 161 Human- and Animal-Pathogenic Pseudomonads

Norberto J. Palleroni

Pseudomonads are widespread in nature and particularly abundant in soil and water, but some are also colonizers or pathogens of plants and animals. Consequently, some species are medically important and frequently can be isolated from a variety of clinical specimens. Most species, however, cause disease in animals and humans only rarely and are classified as opportunistic pathogens.

Chapter 162 Phytopathogenic Pseudomonads and Related Plant-Associated Pseudomonads

Milton N. Schroth, Donald C. Hilderbrand, and Nickolas Panopoulos

(*Pseudomonas fluorescens, P. putida,* and *P. pseudoalcaligenes* are classified as plant pathogens.)

Chapter 164 The Genus *Acinetobacter*

Kevin J. Towner

Acineobacters are ubiquitous organisms that are present in soil, water and sewage. . .

Acinetobacter is a normal inhabitant of human skin. . . Their pathogenicity is generally low, but they may cause occasional serious opportunistic infections. . . Increasing numbers of nosocomial infections due to *Acinetobacter* are now being reported.

APPROVAL

MICROBIOLOGICAL ANALYSIS OF DEBRIS FROM STS-42 IML-1 BY DIRECT PLATING OF RINSE WATERS

By G.A. Smithers

The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

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